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Letter to the Editor

Serum haptoglobin and hemopexin levels are depleted in pediatric sickle cell disease patients

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To the Editor:

Anemia, hemolysis and vaso-occlusion are the hallmarks of sickle cell disease (SCD). The release of hemoglobin (Hb) and heme into the intravascular milieu can promote inflammatory responses including vasculopathy, leukocyte, platelet and endothelial cell activation, thrombosis, and even renal injury [1]. Nature defends the vasculature from hemoglobin/heme by plasma haptoglobin and hemopexin, which tightly bind free hemoglobin and heme, respectively. Haptoglobin-hemoglobin and hemopexin-heme complexes bind to CD163 and CD91 receptors found primarily on macrophages and hepatocytes respectively, and are taken up by receptor-mediated endocytosis [2]. While it is generally accepted that haptoglobin and hemopexin are depleted in SCD patients [3], we found a limited number of publications that have reported human plasma haptoglobin levels in SCD patients [4–11] and only two papers from 1968 and 1971 that have reported human plasma hemopexin levels in SCD patients, albeit in limited numbers [5, 7]. We found no papers that compared hemopexin levels in SS, SC and AA children. In this letter, we examined serum haptoglobin and hemopexin levels and biomarkers of hemolysis in SS, SC and AA children in Brazil.

Table 1
Association of laboratory parameters in SS and SC patients and AA individuals.

	SS patients $N = 179$	SC patients $N = 93$	AA individuals $N = 28$	p value	Dunn's multiple comparisons test		
	Mean ± SE	Mean ± SE	Mean ± SE		SS vs SC	SS vs AA	SC vs AA
Gender, % female	39.88	54.63	51.72				
Age, years	9.77 ± 0.52	10.70 ± 0.93	8.82 ± 0.66				
Hemolysis markers							
RBC, 10^6/mL	2.73 ± 0.03	4.34 ± 0.05	4.71 ± 0.34	< 0.001	< 0.001	< 0.001	0.290
Hemoglobin, g/dL	8.46 ± 0.09	11.45 ± 0.10	12.81 ± 0.17	< 0.001	< 0.001	< 0.001	0.029
Reticulocytes, %	7.28 ± 0.17	3.95 ± 0.18	0.84 ± 0.04	< 0.001	< 0.001	< 0.001	< 0.001
Reticulocytes, 10^4/mL	19.94 ± 0.56	17.44 ± 0.89	3.97 ± 0.22	< 0.001	0.0122	< 0.001	< 0.001
Total bilirubin, mg/dL	2.30 ± 0.08	1.01 ± 0.05	0.49 ± 0.03	< 0.001	< 0.001	< 0.001	0.006
Indirect bilirubin, mg/dL	1.75 ± 0.07	0.70 ± 0.04	0.25 ± 0.02	< 0.001	< 0.001	< 0.001	0.003
LDH, U/L	1231.00 ± 36.70	587.00 ± 20.13	426.30 ± 16.40	< 0.001	< 0.001	< 0.001	0.075
Hemopexin, μg/mL	251.70 ± 17.36	815.70 ± 42.02	2077.00 ± 124.20	< 0.001	< 0.001	< 0.001	0.002
Haptoglobin, μg/mL	49.15 ± 3.47	60.14 ± 6.30	493.70 ± 63.30	< 0.001	0.898	< 0.001	< 0.001
Total heme, μM	80.67 ± 4.96	38.06 ± 1.91	46.45 ± 4.11	< 0.001	< 0.001	0.002	0.472
Leukocytes							
WBC, 10^9/mL	13.00 ± 0.32	8.58 ± 0.28	7.43 ± 0.51	< 0.001	< 0.001	< 0.001	0.436
Platelets							
Platelets, 10^3/mL	418.6 ± 10.86	274.80 ± 11.42	314.70 ± 13.13	< 0.001	< 0.001	0.002	0.344
Lipid metabolism							
Total cholesterol, mg/dL	123.60 ± 1.78	136.20 ± 2.92	163.70 ± 7.21	< 0.001	< 0.001	< 0.001	0.002
HDL-C, mg/dL	32.25 ± 0.65	40.18 ± 0.97	49.96 ± 2.58	< 0.001	< 0.001	< 0.001	0.016
LDL-C, mg/dL	72.93 ± 1.41	79.10 ± 2.26	95.96 ± 6.72	< 0.001	0.070	< 0.001	0.070
VLDL-C, mg/dL	17.26 ± 0.52	15.29 ± 0.63	16.71 ± 1.34	0.048	0.043	0.999	0.821
Inflammation							
CRP, mg/L	5.47 ± 0.33	3.51 ± 0.32	1.28 ± 0.12	< 0.001	< 0.001	< 0.001	< 0.001

SE: Standard error; RBC: Red blood cells; LDH: Lactate dehydrogenase; WBC: White blood cells; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; CRP: C-reactive protein. p value obtained using the Kruskal-Wallis test. Comparisons between groups were obtained using Dunn's multiple comparisons test.

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A cross-sectional study was performed including 272 unrelated steady-state children with SCD; 179 with sickle cell anemia (HbSS) and 93 with hemoglobin SC disease (HbSC), all seen at the Foundation of Hematology and Hemotherapy of the Bahia state (HEMOBA), in Brazil. The study also included 28 healthy children (HbAA) selected from the Clinical Laboratory of the Pharmacy School (Federal University of Bahia). The AA controls were selected and matched to cases by age, gender, and African ethnic origin. All patients were in steady-state that

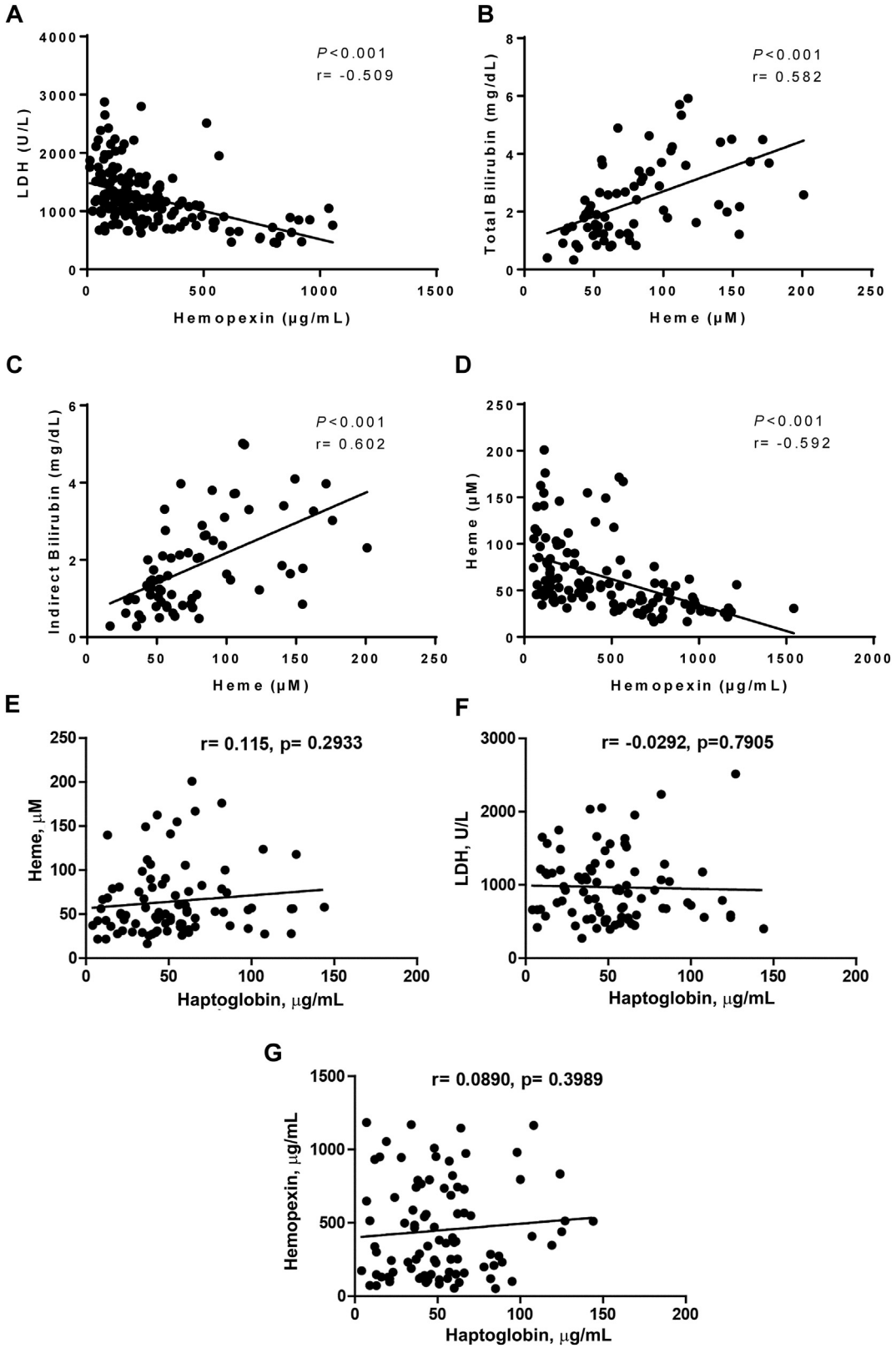

Fig. 1. Correlations of hemopexin, haptoglobin, heme and hemolytic biomarkers in SCD patients. (A) Hemopexin and LDH; (B) heme and total bilirubin; (C) heme and indirect bilirubin; (D) hemopexin and heme; (E) haptoglobin and heme; (F) haptoglobin and LDH; and (G) haptoglobin and hemopexin.

was defined as an absence of any blood transfusion and acute events for a period of four months prior to blood sampling. In addition, exclusion criteria for the study included infectious diseases or inflammatory episodes at the time of blood sampling. This study received approval from the Institutional Research Board of the Oswaldo Cruz Research Foundation and is in compliance with the Declaration of Helsinki 1964 and its subsequent amendments. Since all individuals were under 18 years, their legal guardians signed terms of informed consent.

Hematological parameters were obtained using a Coulter Counter T-890 system hematologic analyzer (Coulter Corporation, Miami, FL, USA) and hemoglobin profiles were analyzed by high-performance liquid chromatography (Bio-Rad, Hercules, California, USA). Biochemical parameters were determined using an A25 immunochemistry analyzer (Biosystems S.A, Barcelona, Catalunya, Spain). Serum hemopexin and haptoglobin levels were investigated by enzyme-linked immunoassay (ELISA, Kamiya Biomedical, Seattle, WA, USA) following the manufacturer's instructions, as well as the plasma concentrations of total heme using the QuantiChrom Heme Assay Kit (Bioassay Systems, Hayward, CA, USA). Statistical analyses were performed using the Statistical Package for the Social Sciences v. 20.0 software (IBM, Armonk, NY, USA). *P* values were obtained using the Kruskal-Wallis test. Comparisons between SS, SC and AA were obtained using Dunn's multiple comparisons test. All data and statistical analyses are presented in Table 1. Spearman's rank correlation (r) was carried out to determine correlations between pairs of variables.

Evaluating the haptoglobin and hemopexin levels, we found that SS and SC patients have lower haptoglobin and hemopexin levels (SS < SC < AA) than AA controls (Table 1). Similarly, SS and SC patients have lower hemoglobin (SS < SC < AA) and increased reticulocyte counts and serum LDH (SS > SC > AA) than AA controls. Total and indirect bilirubin followed a similar pattern (SS > SC > AA). These data suggest more hemolysis in the following pattern: SS > SC > AA.

CRP levels and white blood cell counts are evidence for more inflammation in SCD (SS > SC > AA). Heme levels and platelet counts were higher in SS patients, but similar in SC and AA patients. Finally, Total, HDL, and LDL-cholesterol levels were lower in patients with more hemolysis (SS < SC < AA). Hypocholesterolemia is associated with various forms of anemia [12]. The rapid turnover of red blood cells in SCD patients might reduce plasma cholesterol levels by increasing the demand for cholesterol in newly synthesized reticulocyte membranes.

The correlations between hemopexin, haptoglobin, heme and other markers of hemolysis within SCD patients (SS + SC) are presented in Fig. 1. Hemopexin was negatively correlated to LDH ($r = -0.509$, $p < 0.001$, Fig. 1A) and plasma heme ($r = -0.592$, $p < 0.001$, Fig. 1D). Total plasma heme was positively correlated to total bilirubin ($r = 0.582$, $p < 0.001$, Fig. 1B) and indirect bilirubin ($r = 0.602$, $p < 0.001$, Fig. 1C). Haptoglobin was not correlated to total plasma heme ($r = 0.155$, $p = 0.293$, Fig. 1E) or LDH ($r = -0.0292$, $p = 0.7905$, Fig. 1F). Also haptoglobin and hemopexin were not significantly correlated ($r = 0.0890$, $p = 0.3989$, Fig. 1G). Although both haptoglobin and hemopexin were depleted in SCD patients relative to normal controls, hemopexin was superior to haptoglobin as a marker of hemolysis within the SCD patient subgroup.

Our data demonstrate that serum haptoglobin and hemopexin are depleted in children with SS and SC disease and should be considered as clinical indicators of hemolysis. Recent data in hyperhemolytic sickle mice suggest that haptoglobin and hemopexin supplementation might be beneficial to decrease vascular inflammation and vaso-occlusion in SCD [13].

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Competing interests

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